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Characterization of nuclear microsatellite loci in the calcareous fen specialist *Scorpidium cossonii* (Calliergonaceae)[1]

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- *Premise of the study*: Nuclear microsatellite markers were developed in the fen specialist moss, *Scorpidium cossonii*, to study genetic diversity and genetic structure of this species in relation to land-use types, habitat fragmentation, and habitat conservation measures.
- *Methods and Results*: The polymorphisms of 14 microsatellite markers were characterized. All primers were tested on 140 gametophytes collected from four populations in Switzerland. The primers amplified di- and trinucleotide repeats with three to 26 alleles per locus. The primers worked also in the two other species of the genus: 14 and 12 primers successfully amplified in *S. revolvens* and *S. scorpioides*, respectively.
- *Conclusions*: In future studies, our primers have the potential to provide valuable information on genetic diversity, genetic structure, and on historical and recent gene flow in *S. cossonii*; they should also enable related research in other *Scorpidium* species.

Key words: bryophytes; calcareous fens; cross-species amplification; microsatellites; *Scorpidium cossonii*.

Scorpidium cossonii (Schimp.) Hedenäs is a locally abundant pleurocarpous moss species (Hedenäs, 1989) that is characteristic for calcareous fens. In Central Europe, calcareous fens belong to the most species-rich habitats. Despite legal protection, however, these fens are highly endangered by land-use changes and by disturbances of the hydrology, nutrient enrichment, and habitat fragmentation (Lienert et al., 2002; Peintinger et al., 2003; Bergamini et al., 2009). *Scorpidium cossonii* is a haploid and dioicous moss species (Hedenäs, 1989). Sporophytes are rarely produced, and local population maintenance is presumably mainly by clonal growth. Its vegetative spread, however, seems to be quite restricted, at least in the short term (Hajkova et al., 2009). Nevertheless, the species is widely distributed in northern arctic to temperate regions and at high elevations in the Andes (Hedenäs, 2009).

In this study, we present the first set of polymorphic nuclear microsatellite markers for *S. cossonii*, a species assumed to be suffering from isolation and habitat deterioration. These markers will subsequently be applied to study the effects of habitat fragmentation and land-use types on the genetic diversity at different spatial scales as well as the genetic differentiation between populations of this fen specialist species.

METHODS AND RESULTS

We pooled DNA of *S. cossonii* specimens sampled from six different populations in northern Switzerland. Ecogenics GmbH (Zurich, Switzerland) developed a genetic library from size-selected genomic DNA ligated into an SNX forward/SNX reverse linker (Hamilton et al., 1999) and enriched by magnetic bead selection with biotin-labeled $(CT)_{13}$, $(GT)_{13}$, $(AAC)_{10}$, and $(ATC)_{10}$ oligonucleotide repeats (Gautschi et al., 2000a, 2000b). The enriched library was cloned into chemically competent DH5alpha cells. Of 528 single picked recombinant colonies screened, 341 gave a positive signal after hybridization with the respective biotin-labeled oligonucleotide repeat. Plasmids from 48 positive clones were sequenced and primers were designed for 20 microsatellite inserts, of which 14 were tested for polymorphism.

The microsatellite loci were tested for variability on 140 gametophytes of *S. cossonii* collected from four populations in the pre-Alps of northeastern Switzerland. Total genomic DNA was extracted from stem tissue using the DNeasy 96 Plant Kit (QIAGEN, Hombrechtikon, Switzerland). All forward primers were labeled with one fluorescent dye at the 5′ end region and three PCR multiplexes were set up (Table 1). PCR reactions were performed in a volume of 5 μL containing about 1–5 ng genomic DNA, varying primer concentrations (Table 1), and 1× QIAGEN Multiplex PCR Master Mix (QIAGEN). PCR cycles were started with 15 min at 95°C initial denaturation, followed by 30 cycles of 30 s at 94°C, 90 s at annealing temperature (Table 1), 1 min at 72°C, ending with a final extension step of 60 min at 60°C. PCR products were run on a 3130xl Genetic Analyzer (Applied Biosystems, Foster City, California, USA) using LIZ-500 as internal size standard, and alleles were sized with GENEMAPPER 3.7 (Applied Biosystems). The variability of each microsatellite locus was measured by counting the number of alleles and calculating gene diversity using Arlequin version 3.11 (Excoffier et al., 2005).

Among the 14 microsatellite motifs, nine were dinucleotide repeats, three were trinucleotide repeats, and two were compound repeats containing

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TABLE 1. Characteristics of 14 nuclear microsatellite loci in *Scorpidium cossonii*. For each locus, the forward and reverse primer sequences, repeat motif, labeled fluorescent dye, size of the original fragment, PCR multiplex group, primer concentration, annealing temperature when run in multiplex (T_a), and GenBank accession number are shown.

Locus	Primer sequence (3′–5′)	Repeat motif	Fluorescent dye	Size (bp)	Multiplex	Primer conc. (μM)	T_a (°C)	GenBank Accession No.
Sc01	F: AGCCAAAACGTATGGAAACC	$(AG)_{24}$	FAM	178	1	0.14	59	JF700218
	R: GATAGGAAGGCAGTGCAACC					0.14		
Sc02	F: AAATGGAGTATTGGATGAGATGG	$(AC)_{13}$	NED	159	1	0.21	59	JF700219
	R: CTAATGCAAGGGCATGAGTG					0.21		
Sc03	F: CTCAAGTCACGGCTGCTTC	$(GT)_{16}$	NED	149	2	0.08	59	JF700220
	R: CAAAGGGACTTCAATTCCAAC					0.08		
Sc04	F: TGTGAACGTGGAAGCTTGTG	$(GT)_{17}$	PET	137	1	0.07	59	JF700221
	R: GCCCGAAGTAGTTGCTTCTC					0.07		
Sc07	F: AGGATGAGAGTTCCCTGGTG	$(CA)_{13}$	FAM	205	3	0.08	60	JF700222
	R: TGGCATTCAAACACACATCC					0.08		
Sc09	F: ACGTCAAGTGACCCCACAC	$(AC)_{22}$	VIC	156	1	0.07	59	JF700223
	R: TTGGGTAGTTTGGCGTTAGG					0.07		
Sc13	F: ACAGCAGCACATGTCCAGAG	$(CAA)_9$	PET	143	2	0.08	59	JF700224
	R: GAGGTAATCCAGCAGCCATC					0.08		
Sc16	F: AATCAAGGCCCCATCTAAGC	$(CAT)_{13}$ $(CAA)_4$	FAM	208	2	0.08	59	JF700225
	R: TCACAGATCGCAAACAAACC					0.08		
Sc17	F: GTGTGAACCTGCACGAGATG	$(GTT)_8$ $(GCT)_6$	VIC	191	2	0.08	59	JF700226
	R: CATGGCCGTAAGAGTGGAAC					0.08		
Sc18	F: CTTCAGAAAGCCGATCAAGC	$(CAA)_8$	NED	127	3	0.08	60	JF700227
	R: TTGCGGAAGATCTCCTCTTG					0.08		
Sc19	F: GCGCTTGTATAAGCCTGAGC	$(GTT)_7$	PET	135	3	0.08	60	JF700228
	R: TGCCACTAACGAAGGGACTG					0.08		
Sc20	F: CGCTCTAATTGAGGCATTTG	$(AC)_{20}$	VIC	172	3	0.16	60	JF700229
	R: TGGTTTCGATTTCGGACAAG					0.16		
Sc21	F: TGGAGGCTTGTCCAGAATTG	$(TG)_{13}$	FAM	93	3	0.08	60	JF700230
	R: CGACACTGCAAACCAACTAGAC					0.08		
Sc22	F: GGCATCCCTACCTCATTGACCG	$(CA)_{22}$	FAM	159	2	0.16	59	JF700231
	R: GGGGGCTTTCACCAGGAATTCA					0.16		

two different trinucleotide repeats (Table 1). The microsatellite loci produced three to 26 alleles per locus and mean gene diversities over four populations varied from 0.13 to 0.68 (Table 2). Three individuals of each of two closely related species (Hedenäs, 2009), *S. revolvens* (Sw.) Rubers and *S. scorpioides* (Hedw.) Limpr., were tested for cross-species amplifications. After PCR optimization for the annealing temperature, all 14 primers successfully amplified in *S. revolvens* and 12 primers (except Sc02 and Sc22) in *S. scorpioides*.

CONCLUSIONS

The polymorphic microsatellite loci reported here are a promising tool for investigations of current genetic diversity, genetic structure, and historical as well as recent gene flow in *S. cossonii*. This research will considerably improve our basic understanding of the genetic structure of fragmented bryophyte

TABLE 2. Results of the initial primer screening in four populations of *Scorpidium cossonii*. Shown for each primer are the number of alleles observed (N_A) and gene diversity (H_e). Sample sizes per population and geographical coordinates are given in parentheses.

	Population 1 (N = 35) (47°15′12.48″N, 9°6′3.71″E)		Population 2 (N = 35) (47°8′2.73″N, 8°48′9.22″E)		Population 3 (N = 35) (47°7′41.58″N, 8°55′3.36″E)		Population 4 (N = 35) (47°7′33.27″N, 8°51′3.5″E)		Total	Mean
Locus	N_A	H_e	N_A	H_e	N_A	H_e	N_A	H_e	N_A	H_e
Sc01	9	0.82	11	0.89	7	0.31	6	0.70	26	0.68
Sc02	4	0.66	6	0.58	4	0.31	7	0.67	13	0.55
Sc03	3	0.51	3	0.54	3	0.26	7	0.70	8	0.50
Sc04	4	0.66	4	0.57	6	0.35	3	0.54	9	0.53
Sc07	3	0.64	4	0.39	2	0.05	5	0.62	8	0.42
Sc09	4	0.66	7	0.65	5	0.31	7	0.70	13	0.58
Sc13	2	0.44	1	0.00	2	0.05	2	0.05	3	0.13
Sc16	3	0.62	2	0.48	3	0.25	2	0.05	4	0.35
Sc17	2	0.11	4	0.43	2	0.05	3	0.55	5	0.28
Sc18	3	0.68	3	0.54	3	0.21	4	0.58	5	0.50
Sc19	3	0.65	3	0.54	3	0.25	3	0.58	3	0.50
Sc20	2	0.11	2	0.39	2	0.11	2	0.49	3	0.27
Sc21	3	0.60	3	0.51	3	0.11	2	0.20	3	0.35
Sc22	4	0.75	4	0.56	4	0.21	7	0.70	9	0.55
Mean	3.50	0.57	4.07	0.50	3.50	0.20	4.29	0.51	8.00	0.44
SD	1.74	0.21	2.53	0.19	1.56	0.11	2.13	0.23	6.26	0.24

populations, and it will serve as a base for the development of conservation strategies in this highly specialized fen species. Furthermore, because these loci are applicable to other *Scorpidium* species, they can be widely used and a number of possible studies using these microstellites are in progress.

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APPENDIX 1. Information on voucher specimens from four populations deposited in the Swiss Federal Research Institute WSL. From each population, 35 specimens have been sampled. Because of the very small size of some of the individual plants, the whole plant had to be used for DNA extraction. However, in these cases additional plants from the same patch are kept. Information presented: taxon, voucher specimens, locality in Switzerland.

Scorpidium cossonii: *902-1–902-35*, Höchi, Ebnat-Kappel, Switzerland; *1141-1–1141-35*, Sulzel, Einsiedeln, Switzerland; *1544-1–1544-35*, between Bergweid and Farenegg, Vorderthal, Switzerland; *2344-1–2344-35*, Sattelegg, Vorderthal, Switzerland.